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A novel peptide shows excellent anti-HIV-1 potency as a gp41 fusion inhibitor

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ABSTRACT

Fusion inhibitors of HIV prevent the virus from entering into the target cell via the interaction with gp41, which stops the process of spatial rearrangement of the viral envelope protein. A series of peptides have been designed and screened to obtain a highly potent novel sequence. Among them, CT105 possesses the most potent anti-viral ability at low nanomolar IC50 values against a panel of HIV-1 pseudoviruses from A, B, C and A_1/D subtypes, whereas T20 shows much weaker potency. CT105 also shows excellent inhibitory activity at 260 pico molar IC50 against HIV-1 replication. As a fusion inhibitor, CT105 has a strong ability to interrupt gp41 core formation. The terminal half-life of CT105 possesses 1.72-fold longer than that of T20 as determined by developing an indirect competitive ELISA method. The results suggest that this artificial peptide CT105 could be a favorable architype for further optimization and modification.

The entry of human immunodeficiency virus (HIV) is triggered by recognition between envelope protein (env) on the virions and the CD4 receptor on the cell surface.[1–3] Env is a trimeric glycoprotein composed of three copies of gp120/gp41 heterodimers that originate from precursor protein gp160.[1,4] The inner domain of gp120 interacts with gp41, and its spatial rearrangement results in the dissociation of the gp120–gp41 complex and conformational changes of the gp41 domains in preparation for catalyzing membrane fusion.[5,6] HIV fusion inhibitors are designed to prevent the virus from entering into host cells by blocking the gp41 conformational transition.[7]

Understanding the structure of gp41 at each step during HIV fusion is of fundamental significance in designing fusion inhibitors for preventing further infection. Gp41 is a 345-residue polypeptide from 512 to 856 of *env* according to the HXB2 HIV-1 strain.[8,9] The N-terminal helical heptad repeat region (NHR) and the C-terminal helical heptad repeat region (CHR) form the six helix bundle (6-HB) that represents the gp41 core.[1]

T20 (generic name: Enfuvirtide, brand name: Fuzeon) is a representative peptide fusion inhibitor and the only one that has been put into clinical use.[10] However, its high-dosage injection requirements and rapid plasma clearance rate render it limit in salvage treatment for cocktail resistant patients.[8] Moreover, extended exposure, especially if the virus is not completely restrained, can cause viral resistance to T20.[11,12] T20 could induce a mutant virus with high resistance (81-fold) to T20 in about one month.[13]

Because T20 can induce drug resistance, it is necessary to generate new peptide fusion inhibitors.[14] Our group has previously designed a series of peptides based on the sequence of the CHR of gp41.[15] Briefly, using CP621-652 as a peptide designing template, a series of systematic replacements were introduced to enhance the α-helicity of synthesized peptides that correlates with the anti-HIV activity. Charged residues were incorporated at favorable potentials for intra-helical salt-bridges, and alanine was introduced to promote helix formation. Initial screening showed CT105 as the most potent fusion inhibitor.

Molecular docking research has found that CT105 could wrap antiparallel to the trimetric N-terminal heptad repeat (NHR) of gp41 in a left-handed direction.[15] It was suggested that six residues of CT105 (Met626, Thr627, Trp628, Trp631, Glu634 and Tyr638) might insert into the hydrophobic pocket of NHR.[15] The core structure of gp41 and the binding pattern between CT105 and gp41 are presented in Fig. 1. The results of clear native polyacrylamide gel electrophoresis (CN-PAGE) showed the strong binding affinity of CT105 towards N36, a peptide that represents gp41 NHR. Interestingly, the binding mode seems rather complicated. As showed in Fig. 2(A), N36 could not migrate into the gel in CN-PAGE condition because of its positive charge properties. More than four

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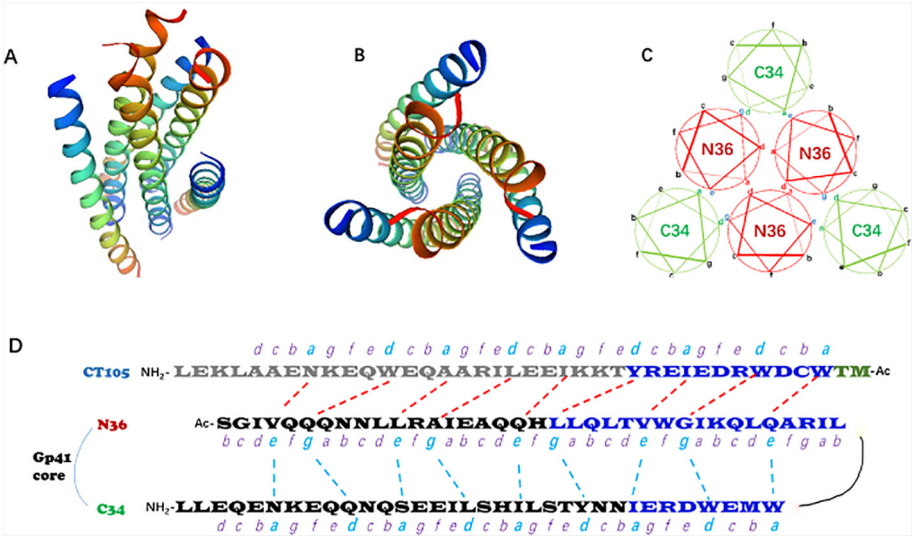

Fig. 1. The core structure of gp41 and CT105. (A) Lateral, (B) axial and (C) helical wheel representations of the 6-HB of gp41 (PDB 1AIK) formed by the N36 and C34 peptides. (C) Heptad-repeat positions are labeled (a) through (g). Residues at the (a), and (d) (shown in red) positions of three NHR interact with each other and constitute the central trimeric coiled-coil. The (a) and (d) residues in the CHR (shown in green) interact with the (e) and (g) sites on the central NHR coiled-coil (shown in blue), which constitutes a hydrophobic core of the trimer-of-hairpins. (D) Sequence of N36, C34 and CT105 and their binding sites.

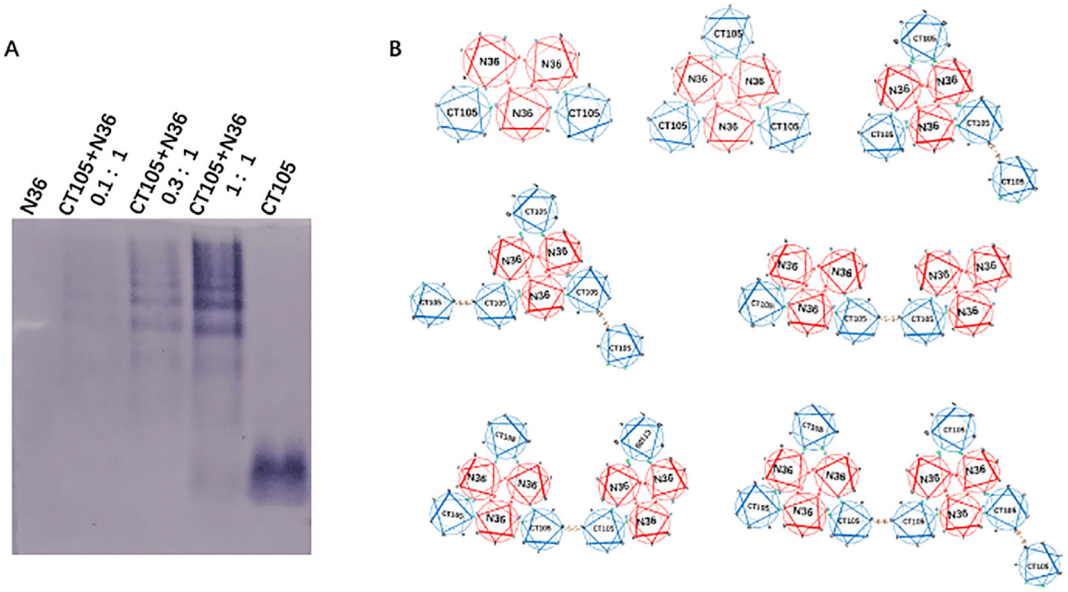

Fig. 2. (A) The CN-PAGE analysis of the interaction between HIV-1 gp41 N36 and CT105. Peptides were incubated at 37 °C for 30 min and then loaded into the polyacrylamide gel (12% separating gel, 4%stacking gel). Electrophoresis was carried out with 120 V constant voltage for 2 h. (B) Possible binding modes formed by N36, CT105 and CT105 dimers.

complexes could be found after incubation. When the N36 peptide was incubated with CT105, several bands appeared that represent the N36-CT105 complex. This indicates that not only 6-HB but also other unknown polymers are presented (see in Fig. 2). We speculate that CT105 might work as a dimer on account of the self-connection of its cysteine. During the incubation process, the CT105 moieties might suffer from slight oxidation that could cause the formation of the disulfide bond. That might be the main reason why so many different complexes are formed. We also observed the interaction among N36, CT105 and C34, a peptide that could form the 6-HB core of gp41 with N36. As presented in Fig. 3, N36 was mixed with C34 peptide at a 1:1 ratio. CT105, at different final concentrations of 5 μM, 15 μM, 25 μM and 50 μM, was incubated with the mixture of C34 and N36. Results showed that CT105 rather than C34 had strong affinity towards N36. CT105 could bind with N36 in a dose-independent manner. The CT105-N36 complex formed when the concentration of CT105 was 10-fold less than that of C34. In addition, C34 exhibited little effect on CT105-N36 disassociation, even if the C34 peptide concentration was high.

To test the inhibitory potency of CT105 against HIV-1, TZM-bl cells were infected with a panel of different subtypes of HIV-1 pseudoviruses. The TZM-bl cells were derived from Hela cells, which express CD4 and, CXCR4 as well as CCR5 receptor, and contain the firefly luciferase, LacZ reporter under the control of a Tat-responsive reporter for quantification.[16] The peptides were prepared with six series of dilutions in a 4-fold stepwise manner, and added to TZM-bl cells together with 100TCID50 of each subtype of HIV-1 pseudoviruses. After 48 h incubation at 37 °C, the